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Do food bacteria use radicals to kill each other?

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Objectives

- To detect radicals formed in pure and mixed cultures of bacteria
- To investigate the role of radicals in interaction between probiotic and pathogenic bacteria

Introduction

Food may contain both probiotic bacteria and bacteria with negative health effects, and in this study radical formation by a strain of *Lactobacillus acidophilus* with well-known probiotic properties and a strain of *Listeria innocua*, used as a model of the human pathogenic strain *Listeria monocytogenes*, was investigated.

Radicals are highly reactive molecules containing an unpaired electron, which may be formed during common metabolic processes. Radicals may serve as a way of communicaton between cells, but are also prone to induce oxidative damage in cells. Radical formation by one bacteria strain could therefore induce the death of another due to oxidative stress.

Detection of radicals can be performed directly with electron spin resonance (ESR) spectroscopy. However, many radicals are very short-lived and in order to detect these, a spin trap can be used, which will allow indirect detection of the radicals. In the present study, the spin trap N-tert-butyl-α-phenylnitrone (PBN) was used for radical detection.

Results and discussion

Radicals were detected in both pure cultures and mixtures using ESR spectroscopy and spin trapping with PBN. In pure cultures of *L. innocua* relatively low signal intensities was observed, whereas pure cultures of *L. acidophilus* and mixtures showed higher intensities (Fig. 1a). Addition of 10% (v/v) ethanol caused an increase in signal intensity in samples containing *L. acidophilus*, whereas the intensity of the signals from pure *L. innocua* decreased slightly (Fig. 1b). An example ESR spectra and the development in intensity over time is shown in Fig. 2, with indication of the peak used for determination of peak height.

The viability of *L. acidophilus* was the same when mixed with *L. innocua* as in pure culture, and addition of 10% (v/v) ethanol had a bacteriostatic effect on *L. acidophilus*. For *L. innocua* the viability was severely affected by mixing with *L. acidophilus*, as this led to the death of *L. innocua* within 8 hours in samples with 2% (v/v) ethanol and within 4 hours in samples containing 10% (v/v) ethanol; 10% (v/v) ethanol was bactericidal towards pure *L. innocua* over 8 hours (Fig. 1, c & d).

These results show that *L. acidophilus* was the main radical producer in the investigated samples, and neither radical formation nor viability of this strain was affected by mixing with *L. innocua*. Furthermore, an antagonistic effect of *L. acidophilus* against *L. innocua* was observed, and is suggested to involve radicals. The detection of radicals adds a possible mechanism of antagonism to the list of known strategies of antagonism (e.g. bacteriocins, organic acids, pH lowering, H_2O_2 production), some of which may also have contributed to the observed effect in the present study.

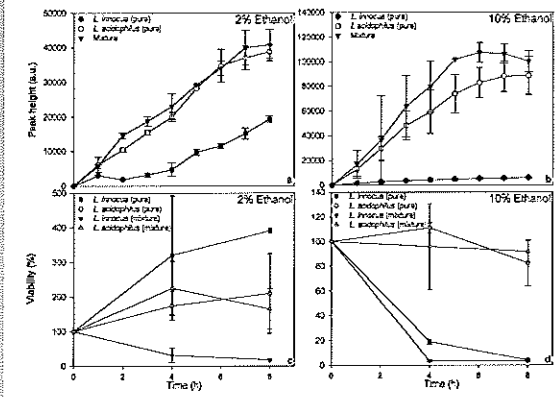

Figure 1: ESR signal peak heights and viabilities of *L. innocua* and *L. acidophilus* in pure and mixed cultures. a & b: ESR peak heights in pure and mixed cultures with 2% and 10% (v/v) ethanol, respectively. c & d: viability of *L. innocua* and *L. acidophilus* in samples with 2% and 10% (v/v) ethanol, respectively.

Conclusions

- Radicals are formed in both pure and mixed cultures of *L. acidophilus* and *L. innocua*, strongest by *L. acidophilus*.
- 2% (v/v) ethanol does not negatively affect viability of the bacteria; 10% (v/v) ethanol is bacteriostatic towards *L. acidophilus* and bactericidal towards *L. innocua*.
- An antagonistic effect of *L. acidophilus* against *L. innocua* is seen and radicals are suggested to be involved as a new mechanism of antagonism.

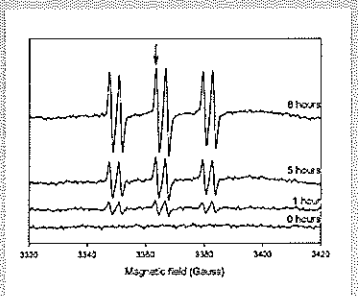

Figure 2: ESR spectra showing development in signal intensity over time in a sample containing *L. innocua* and 2% (v/v) ethanol. The arrow indicates the peak used for peak height determination (Figure 1).

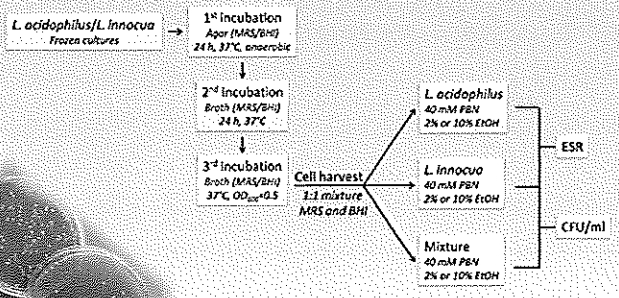

Figure 3: Experimental outline

Experimental

The experiments were conducted as outlined in Figure 3. *L. acidophilus* was grown in and counted on de Man-Rogosa-Sharpe (MRS) medium and *L. innocua* was grown in and counted on brain heart infusion (BHI) medium. Mixtures were counted on both media. ESR measurements were performed at room temperature in hourly intervals from 0 h to 8 h from sample preparation. The samples were incubated at 37°C during the experiments. Counting of bacteria was performed as determination of colony forming units (CFU/ml) after 0, 4 and 8 h from sample preparation.

Viability % of bacteria was calculated as $(N_t/N_0)\cdot 100\%$, where N_t is CFU/ml at time t h and N_0 is CFU/ml at time 0 h. All samples were prepared in biological duplicate and in a completely randomized setup.

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